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An integrated strategy for quality control of the multi-origins herb medicine of Gentianae Macrophyllae Radix based on UPLC-Orbitrap-MS/MS and HPLC-DAD†

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Gentianae Macrophyllae Radix, the dried root of *Gentiana macrophylla* Pall., *Gentiana crassicaulis* Duthie ex Burk., *Gentiana straminea* Maxim., or *Gentiana dahurica* Fisch., is a traditional Chinese medicine with multi-origins and some adulterants. Liquid chromatography coupled to electrostatic orbitrap high-resolution mass spectrometry (LC-Orbitrap-MS) was used to search the different components of Gentianae Macrophyllae Radix of the four species. High-performance liquid chromatography (HPLC) combined with fingerprint analysis, principal components analysis (PCA), and partial least-squares discrimination analysis (PLS-DA) was also utilized to distinguish them and their adulterants based on the critical components identified by LC-MS. A single standard to determine the multi-components (SSDMC) method was established for the determination of the critical markers. A total of 93 compounds were identified from Gentianae Macrophyllae Radix, including 58 common ones. Their HPLC fingerprints show a significant difference with the adulterants. In addition, PCA and PLS-DA could make a distinction among the four species. Loganic acid, 6′-*O*-β-D-glucosylgentiopicroside, swertiamarine, gentiopicroside, and sweroside were identified as the critical markers and then quantified by the SSDMC method. The developed strategy is powerful for the quality control and authentication of Gentianae Macrophyllae Radix.

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1. Introduction

Gentianae Macrophyllae Radix, the dried root of *Gentiana macrophylla* Pall., *Gentiana crassicaulis* Duthie ex Burk., *Gentiana straminea* Maxim., or *Gentiana dahurica* Fisch., has been used as a medicine since Han Dynasty (202 BC to 220 AD) to dispel wind-damp, clear damp-heat, ease pain, and eliminate deficiency-heat.[1–4] They are cultivated in different geographic regions in China and are generally known as Qinjiao (QJ), CuJing Qinjiao (CJQJ), MaHua Qinjiao (MHQJ), and Xiao Qinjiao (XQJ) in Chinese due to their different appearances, respectively (Fig. 1).[1,5–7] Numerous studies have proved that this herbal medicine is abundant in iridoids and secoiridoids, such as loganic acid and gentiopicroside, which have been recorded as quality markers in the Chinese Pharmacopoeia (2020 edition).[3,8] Loganic acid, 6′-*O*-β-D-glucosylgentiopicroside, swertiamarine, gentiopicroside, and sweroside are reported to have various excellent activities.[9–13] Studies have shown that the content of active components of the herb medicine will affect their pharmacological activities, which was the reason for the differences in activity among the Gentianae Macrophyllae Radix of the four species.[14–17]

In addition, there are some adulterants used as Gentianae Macrophyllae Radix in the market (Table 1 and Fig. 1), such as Long dan (the rhizome of *Gentiana scabra* Bge.), Hong qin jiao (the root of *Salvia Przewalskii* Maxim.), and Ma bu qi (the root of *Aconitum sinomontanum* Nakai.). Therefore, the identification of different species and authentication are of great importance for the safety and effectiveness of Gentianae Macrophyllae Radix in clinical practice.

Liquid chromatography coupled to electrostatic orbitrap high-resolution mass spectrometry (LC-Orbitrap-MS) has the advantages of high resolution, quality accuracy,[18] and qualitative analysis of constituents by the in-house and online database. Due to its stability and controllability, high-performance liquid chromatography (HPLC) is still the classic technology for quality control of herbal medicines in pharmacopeia worldwide.

In this paper, the major constituents of Gentianae Macrophyllae Radix were analyzed by LC-Orbitrap-MS. Subsequently, variable influence on projection (VIP) score, K-means

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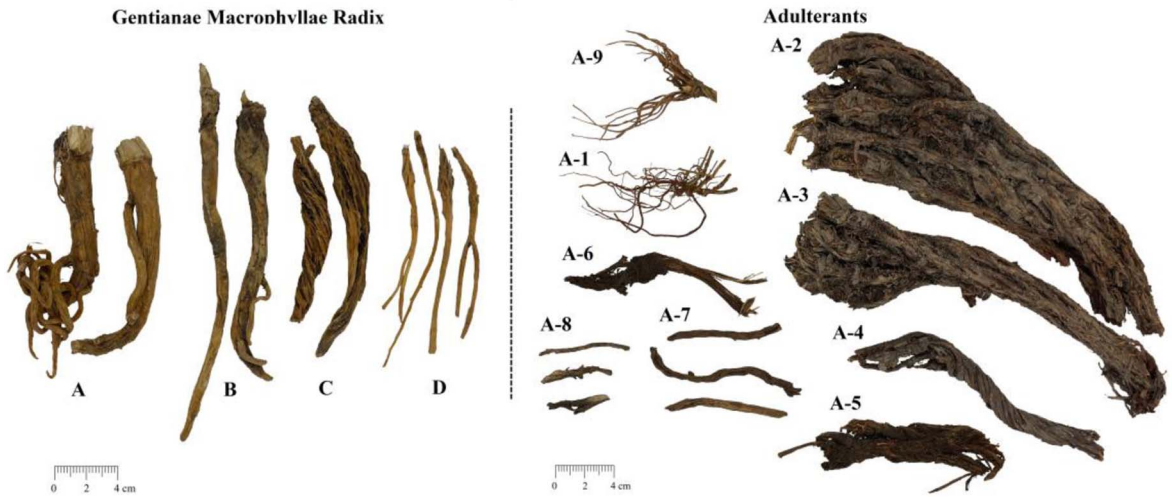

Fig. 1 Gentianae Macrophyllae Radix and the adulterants (A: *Gentiana macrophylla* Pall.; B: *Gentiana crassicaulis* Duthie ex Burk.; C: *Gentiana straminea* Maxim.; D: *Gentiana dahurica* Fisch.).

Table 1 The sample information collected in this study

Sample name	Medicine	Origin	Sample name	Medicine	Origin	Sample name	Medicine/chinese name	Origin/plant
QJ-1	QJ	Yunnan	QJ-15	CJQJ	Sichuan	QJ-29	XQJ	Inner Mongoria
QJ-2	QJ	Yunnan	QJ-16	CJQJ	Sichuan	QJ-30	XQJ	Qinghai
QJ-3	QJ	Yunnan	QJ-17	MHQJ	Xinjiang	QJ-31	XQJ	Inner Mongoria
QJ-4	QJ	Yunnan	QJ-18	MHQJ	Sichuan	QJ-32	XQJ	Inner Mongoria
QJ-5	QJ	Yunnan	QJ-19	MHQJ	Qinghai	QJ-33	XQJ	Xinjiang
QJ-6	QJ	Yunnan	QJ-20	MHQJ	Sichuan	A-1	Long dan	*Gentiana scabra* Bge.
QJ-7	QJ	Yunnan	QJ-21	MHQJ	Qinghai	A-2	Hong qin jiao	*Salvia Przewalskii* Maxim.
QJ-8	CJQJ	Yunnan	QJ-22	MHQJ	Sichuan	A-3	Hong qin jiao	*Salvia Przewalskii* Maxim.
QJ-9	CJQJ	Yunnan	QJ-23	MHQJ	Sichuan	A-4	Hong qin jiao	*Salvia Przewalskii* Maxim.
QJ-10	CJQJ	Sichuan	QJ-24	MHQJ	Sichuan	A-5	Hong qin jiao	*Salvia Przewalskii* Maxim.
QJ-11	CJQJ	Sichuan	QJ-25	MHQJ	Sichuan	A-6	Ma bu qi	*Aconitum sinomontanum* Nakai.
QJ-12	CJQJ	Sichuan	QJ-26	MHQJ	Tibet	A-7	Du yi wei	*Lamiophlomis rotata* (Benth.) Kudo
QJ-13	CJQJ	Qinghai	QJ-27	MHQJ	Sichuan	A-8	Bai tou wen	*Pulsatilla chienensis* (Bge.) Regel
QJ-14	CJQJ	Sichuan	QJ-28	MHQJ	Qinghai	A-9	Bai wei	*Cynanchum atratum* Bunge.

calculation, and self-organizing map (SOM) were used to obtain differentials for the compounds, which were then quantified by HPLC and a single standard for determination of multiple components (SSDMC) method.[19–21] Finally, the fingerprint analysis, principal components analysis (PCA), and partial least-squares discrimination analysis (PLS-DA) were performed to distinguish QJ, CJQJ, MHQJ, and XQJ, as well as the adulterants.

2. Materials and methods

2.1. Reagents and materials

The reference standards, including loganic acid, swertiamarine, gentiopicroside, and sweroside, were purchased from Nature-Standard (Shanghai, China), while 6′-*O*-β-D-glucosylgentiopicroside was supplied by Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). HPLC-grade methanol, acetonitrile, and formic acid were purchased from Sigma-Aldrich (Sigma-Aldrich, Co., Louis, USA). All aqueous solution was prepared with purified water from C'estbon (Shenzhen, China).

Thirty-three batches of Gentianae Macrophyllae Radix (including 7 batches of QJ, 9 batches of CJQJ, 12 batches of MHQJ, and 5 batches of XQJ) were collected from Xinjiang, Yunnan, Tibet, Sichuan, Inner Mongolia, and Qinghai. In addition, nine batches of its adulterants were also collected from different provinces. The details are summarized in Table 1. All the samples were authenticated by Professor Wei Wang (School of Pharmacy, Hunan University of Chinese Medicine), according to the plant morphology. Voucher specimens (QJ 1 ~ 33, Long dan, Hong qin jiao-1 ~ 4, Ma bu qi, Du yi wei, Bai tou wen, and Bai wei) were deposited at the TCM and Ethnomedicine Innovation & Development International Laboratory, Innovative Material Medical Research Institute, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, China.

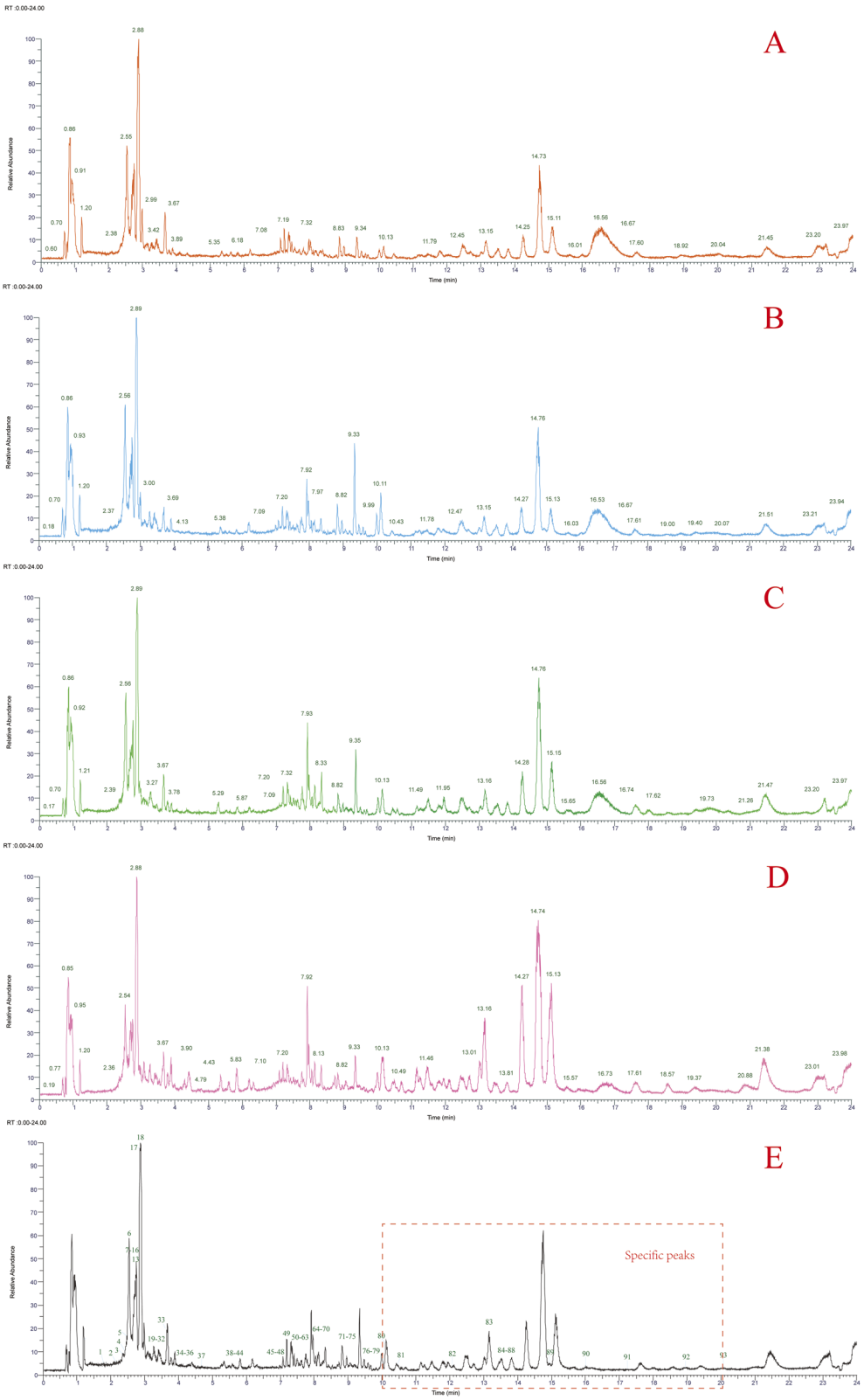

Fig. 2 TIC chromatogram of QJ (A), CJQJ (B), MHQJ (C), XQJ (D), and quality control sample (E).

2.2. Sample preparation

The air-dried roots were pulverized and passed through a 50-mesh sieve. Then, 0.2 g of the powder was accurately weighed and ultrasonic-extracted with 20 mL methanol for 30 min. After 10 min centrifugation at 13 000 rpm and filtration with a 0.22 μm of filter membrane, the sample solution was collected. The

Table 2 The compounds identified by LC-MS

Peak no.	RT (min)	Reference ion	*m/z*	Diff. (ppm)	Formula	Fragment ions (*m/z*)	Identification	QJ	CJQJ	MHQJ	XQJ
1	1.886	$[M-H]^-$	421.1351	−0.11	$C_{17}H_{26}O_{12}$	375.12955, 310.12399, 255.08681, 229.04193, 213.07692, 169.05067, 125.0245	Lamiide	√	√		√
2	2.176	$[M-H]^-$	315.0724	0.78	$C_{13}H_{16}O_9$	272.01117, 259.91162, 229.02588, 180.87544, 165.01967, 153.01947, 109.02966	Gentisic acid-5-*O*-β-glucoside	√	√		
3[a]	2.377	$[M+FA-H]^-$	391.12454	−0.35	$C_{15}H_{22}O_9$	246.82138, 229.03535, 211.06192, 183.06647, 167.07111, 137.06094, 121.06605	Aucubin	√	√	√	√
4	2.439	$[M-H]^-$	375.12949	−0.4	$C_{16}H_{24}O_{10}$	229.03514, 213.07697, 169.08714, 151.0766, 125.06094	Mussaenosidic acid	√	√		√
5	2.499	$[M-H]^-$	188.03529	−0.12	$C_{10}H_7NO_3$	181.76221, 159.87849, 144.04565, 116.05076, 89.20306, 81.13036	Kynurenic acid	√			
6[a]	2.544	$[M-H]^-$	375.12921	−0.63	$C_{16}H_{24}O_{10}$	310.93152, 273.17215, 229.03088, 213.077, 169.08717, 151.07664, 125.06109	Loganic acid	√	√	√	√
7[a]	2.584	$[M+FA-H]^-$	725.21478	0.25	$C_{28}H_{40}O_{19}$	415.73318, 383.1196, 323.09967, 229.04402, 221.06702, 179.0564, 149.06079, 131.03517	Scabrans G3	√	√	√	√
8[a]	2.601	$[M-H]^-$	373.11379	−0.32	$C_{16}H_{22}O_{10}$	364.96799, 229.02231, 211.06148, 193.05083, 167.07156, 149.06096, 123.04534	Geniposidic acid	√	√	√	√
9[a]	2.607	$[M-H]^-$	593.15125	0.09	$C_{27}H_{30}O_{15}$	503.12003, 473.10944, 375.12659, 311.05649, 282.05389, 229.02704, 213.0777	Vicenin-2	√	√	√	√
10[a]	2.65	$[M-H]^-$	405.13995	−0.6	$C_{17}H_{26}O_{11}$	281.06702, 243.09027, 229.04758, 221.0452, 197.08226, 179.03546, 155.03568, 141.05588	Shanzhiside methyl ester	√	√	√	√
11	2.682	$[M+FA-H]^-$	729.26111	0.17	$C_{32}H_{44}O_{16}$	383.11844, 359.1492, 329.13965, 310.11627, 139.07669, 101.02464	(+)-Lariciresinol-4,4′-*O*-β-D-diglucopyranoside			√	√
12[a]	2.707	$[M+FA-H]^-$	563.16179	0.09	$C_{22}H_{30}O_{14}$	374.97791, 304.93506, 229.04768, 221.06778, 193.04979, 179.05632, 149.06085	6′-*O*-β-D-glucosylgentiopicroside	√	√	√	√
13[a]	2.754	$[M+FA-H]^-$	419.11919	−0.65	$C_{16}H_{22}O_{10}$	361.99875, 229.04565, 169.6095, 149.06107, 141.01965	Swertiamarin	√	√	√	√
14	2.791	$[M-H]^-$	403.12551	1.13	$C_{17}H_{24}O_{11}$	273.46982, 249.06258, 229.02711, 195.06598, 179.05597, 161.04498, 153.01945, 149.06076	Gardenoside			√	
15[a]	2.798	$[M-H]^-$	681.23997	1.3	$C_{32}H_{42}O_{16}$	519.13965, 501.16895, 381.1348, 357.13501, 339.12378, 323.07669, 309.11362, 229.02699, 203.07179, 179.05647	Pinoresinol diglucoside	√	√	√	√
16[a]	2.832	$[M+FA-H]^-$	639.15704	0.64	$C_{27}H_{30}O_{15}$	519.11658, 477.10452, 459.09302, 433.11359, 323.07831, 315.0726, 283.26459, 229.05029, 153.01952	Saponarin	√	√	√	√

Table 2 (*Contd.*)

Peak no.	RT (min)	Reference ion	*m/z*	Diff. (ppm)	Formula	Fragment ions (*m/z*)	Identification	QJ	CJQJ	MHQJ	XQJ
17	2.867	$[M-H]^-$	447.09268	−1.34	$C_{21}H_{20}O_{11}$	429.08356, 357.06143, 327.05136, 297.04062, 285.0405, 269.10281, 229.03467, 161.04578	Isoorientin		√	√	√
18[a]	2.881	$[M+FA-H]^-$	401.10856	−1.04	$C_{16}H_{20}O_9$	295.08347, 235.06143, 229.02054, 193.05118, 175.04045, 149.06096, 121.06655	Gentiopicroside	√	√	√	√
19[a]	2.984	$[M-H]^-$	357.11896	0.66	$C_{16}H_{22}O_9$	269.11877, 259.0976, 229.03552, 195.0313, 177.05588, 153.01932, 133.06612	Sweroside	√	√	√	√
20	3.026	$[M+FA-H]^-$	581.1883	1.58	$C_{26}H_{32}O_{12}$	373.12875, 355.11829, 343.1185, 313.1084, 229.02733, 209.08191, 193.0506, 163.04053, 151.04045, 137.02448	8-Hydroxypinoresinol-4′-*O*-β-D-glucopyranoside	√	√		
21[a]	3.062	$[M-H]^-$	431.09847	0.24	$C_{21}H_{20}O_{10}$	413.0878, 387.07269, 341.06653, 327.05112, 311.0564, 283.05881, 255.06628, 229.04552, 205.01439, 178.99817	Isovitexin	√	√	√	√
22	3.083	$[M-H]^-$	359.13467	−0.15	$C_{16}H_{24}O_9$	271.37622, 232.08966, 229.03514, 197.08215, 153.09233, 135.08171, 109.06606, 119.03494	7-Deoxyloganic acid	√	√	√	
23[a]	3.093	$[M-H]^-$	521.20322	0.75	$C_{26}H_{34}O_{11}$	477.13144, 329.13983, 325.05759, 229.02888, 192.07962, 178.06396, 175.07663	Lariciresinol-4-*O*-glucoside	√	√	√	√
24[a]	3.278	$[M-H]^-$	417.15476	−1.74	$C_{22}H_{26}O_8$	402.13223, 387.10843, 236.06927, 229.04073, 190.0636, 181.05084, 166.02734, 152.04797	(+)-Syringaresinol	√	√	√	√
25[a]	3.28	$[M-H+HAc]^-$	579.20863	0.69	$C_{26}H_{32}O_{11}$	357.13419, 342.11081, 311.12927, 229.04831, 151.04018, 135.04543	(−)-Pinoresinol glucoside	√	√	√	√
26[a]	3.333	$[M-H]^-$	191.03512	0.72	$C_{10}H_8O_4$	176.04822, 163.95222, 147.04532, 144.86652, 111.00893, 87.00882	6,7-Dihydroxy-4-methylcoumarin	√	√	√	√
27[a]	3.404	$[M-H]^-$	609.18261	0.21	$C_{28}H_{34}O_{15}$	503.62744, 488.21753, 367.08380, 343.082, 325.07181, 301.07217, 286.04901, 257.08224, 229.04509, 179.78441, 125.02504	Hesperidin	√	√	√	√
28	3.452	$[M+FA-H]^-$	569.15155	0.16	$C_{24}H_{28}O_{13}$	476.10995, 474.09473, 388.09415, 374.32324, 289.98453, 229.04309, 137.02455, 93.03461	(+)-Seguinoside D			√	√
29[a]	3.473	$[M-H]^-$	397.11412	0.34	$C_{18}H_{22}O_{10}$	356.28165, 328.25037, 326.10419, 235.0983, 229.04501, 153.01874, 149.06119	6′-*O*-Acetyl-gentiopicroside	√	√	√	√
30[a]	3.483	$[M+FA-H]^-$	417.21305	0.1	$C_{19}H_{32}O_7$	349.17096, 252.32874, 229.04927, 161.04509, 141.23784, 123.11726	Blumel C glucoside	√	√	√	√

Table 2 (*Contd.*)

Peak no.	RT (min)	Reference ion	*m/z*	Diff. (ppm)	Formula	Fragment ions (*m/z*)	Identification	QJ	CJQJ	MHQJ	XQJ
31	3.485	$[M-H]^-$	697.19905	0.69	$C_{31}H_{38}O_{18}$	655.1897, 571.16766, 535.14624, 475.12473, 409.11395, 367.10291, 349.09293, 315.07251, 229.03539, 153.01949	Gentistraminoside A			√	√
32[a]	3.571	$[M-H]^-$	447.0936	0.82	$C_{21}H_{20}O_{11}$	357.06213, 327.05191, 285.04074, 229.03545, 177.01965, 116.92889	Kaempferol-7-*O*-glucoside	√	√	√	√
33	3.658	$[M-H]^-$	521.16652	0.31	$C_{25}H_{30}O_{12}$	359.1142, 357.11865, 315.12424, 297.11343, 229.0264, 213.07632, 195.06635, 163.04022, 151.07657	2′-*O*-(4″-Hydroxycinnamoyl)-mussaenosidic acid			√	√
34	3.993	$[M-H]^-$	755.2045	0.64	$C_{33}H_{40}O_{20}$	713.19714, 613.17859, 593.15192, 571.16803, 533.12988, 451.12473, 409.11401, 391.10428, 367.10297, 349.09274, 315.07257, 229.0226, 153.01955	Gentistraminoside B			√	√
35	4.3	$[M-H]^-$	479.15596	0.44	$C_{23}H_{28}O_{11}$	357.11929, 273.11514, 234.43077, 229.03462, 195.0663, 151.07655, 121.02967	Albiflorin			√	
36[a]	4.306	$[M+FA-H]^-$	493.22922	0.43	$C_{21}H_{36}O_{10}$	315.18182, 285.11374, 229.05032, 191.05629, 161.04556, 131.03499, 113.02454, 101.02451	Atractyloside A	√	√	√	√
37[a]	4.692	$[M-H]^-$	301.03555	0.57	$C_{15}H_{10}O_7$	286.04874, 257.04626, 242.0584, 233.0457, 193.01447, 164.01154, 151.00389, 125.02461	Quercetin	√	√	√	√
38	5.234	$[M-H]^-$	301.14468	0.48	$C_{18}H_{22}O_4$	283.13483, 257.15469, 229.03175, 213.16476, 193.01418, 177.09232, 149.81148, 106.89589	Terbucromil	√		√	√
39[a]	5.272	$[M-H]^-$	797.21476	0.21	$C_{35}H_{42}O_{21}$	755.20563, 655.18994, 635.16437, 613.17889, 593.15143, 493.13611, 451.12488, 409.11401, 315.07251, 153.01958	Rindoside	√	√	√	√
40[a]	5.385	$[M-H]^-$	269.04572	0.64	$C_{15}H_{10}O_5$	225.0562, 200.88235, 181.91168, 159.04614, 151.00385, 117.03601	Aloe-emodin	√	√	√	√
41	5.55	$[M-H]^-$	299.05627	0.54	$C_{16}H_{12}O_6$	284.0329, 256.03848, 229.02341, 190.84854, 169.60602, 134.90634	Hispidulin	√	√	√	
42[a]	5.615	$[M-H]^-$	285.04067	0.74	$C_{15}H_{10}O_6$	257.04556, 241.05099, 199.04027, 193.01436, 177.0195, 151.00386, 133.02988	Luteolin	√	√	√	√
43	5.847	$[M-H]^-$	781.21987	0.26	$C_{35}H_{42}O_{20}$	697.20068, 655.1911, 619.16736, 577.15662, 493.13516, 451.12427, 315.07239, 153.0195	Trifloroside			√	√
44	6.087	$[M-H]^-$	955.49086	0.08	$C_{48}H_{76}O_{19}$	835.44794, 793.4389, 731.43817, 613.37518, 569.38464, 523.37915, 455.35229, 229.04059	Gensenoside Ro		√		

Table 2 (*Contd.*)

Peak no.	RT (min)	Reference ion	*m/z*	Diff. (ppm)	Formula	Fragment ions (*m/z*)	Identification	QJ	CJQJ	MHQJ	XQJ
45[a]	6.782	$[M-H]^-$	519.33282	0.11	$C_{30}H_{48}O_7$	501.32266, 453.3027, 451.28586, 435.28998, 389.28601, 365.28641, 229.04555, 152.99568	Cucurbitacin P	√	√	√	√
46	6.833	$[M-H]^-$	793.43851	0.67	$C_{42}H_{66}O_{14}$	733.41687, 673.39227, 631.38538, 613.37506, 569.38501, 455.3537, 356.71674, 317.46048, 229.02242, 175.02512, 157.01413	Fatsiaside C		√		
47	6.898	$[M-H]^-$	821.39696	0.54	$C_{42}H_{62}O_{16}$	759.39392, 645.37128, 627.3584, 351.05676, 333.04721, 289.0556, 229.04875, 193.03555	Glycyrrhizic acid		√		
48	6.989	$[M-H]^-$	319.1188	0.27	$C_{17}H_{20}O_6$	287.09247, 275.12903, 243.10214, 207.06651, 205.05106, 191.03516, 179.03526, 148.05307	Mycophenolic acid		√		√
49[a]	7.2	$[M-H]^-$	325.20226	0.62	$C_{18}H_{30}O_5$	307.19183, 289.18204, 263.20181, 229.03473, 195.10229, 171.1026, 151.11298, 137.09737, 125.09727, 111.08197	2,3-Dinor-11-β-prostaglandin F2α	√	√	√	√
50[a]	7.265	$[M-H]^-$	485.32726	0.13	$C_{30}H_{46}O_5$	407.29648, 373.28903, 273.11261, 231.10339, 193.05061, 179.03552, 155.53018, 111.57957	(3β,4)-3,23-Dihydroxy-1-oxoolean-12-en-28-oic acid	√	√	√	√
51[a]	7.311	$[M+FA-H]^-$	549.34344	0.11	$C_{30}H_{48}O_6$	470.01324, 441.30182, 393.09708, 349.1084, 285.0416, 229.04987, 193.01363, 111.00887	Arjungenin	√	√	√	√
52[a]	7.329	$[M-H]^-$	299.05623	0.4	$C_{16}H_{12}O_6$	284.03311, 271.06165, 240.04305, 207.03024, 191.03517, 176.01167, 165.01964, 139.0403, 133.02956	Kaempferide	√	√	√	√
53	7.4	$[M-H]^-$	373.16547	−0.51	$C_{21}H_{26}O_6$	355.15488, 329.17667, 285.18646, 246.0903, 229.03528, 191.03514, 178.02711	Ustosolate E		√		
54[a]	7.407	$[M-H]^-$	487.34297	0.17	$C_{30}H_{48}O_5$	373.74661, 251.06982, 229.02534, 86.7402	Asiatic acid	√	√	√	√
55[a]	7.412	$[2M-H]^-$	499.30671	0.47	$C_{15}H_{22}O_3$	229.03546, 205.16005, 189.12825, 163.00533, 141.0988, 121.06618, 116.97608	2-[(1*S*,2*S*,4*aR*,8*aS*)-1-Hydroxy-4*a*-methyl-8-methylidene-decahydronaphthalen-2-yl]prop-2-enoic acid	√	√	√	√
56	7.536	$[M-H]^-$	503.33804	0.85	$C_{30}H_{48}O_6$	490.36255, 301.03766, 247.0618, 229.04099, 193.01413, 152.99648, 116.92847	Sericic acid	√			
57[a]	7.656	$[M-H]^-$	269.04576	0.8	$C_{15}H_{10}O_5$	251.20149, 229.0273, 211.13416, 197.15456, 185.11882, 150.9537, 130.08713, 119.43418	Apigenin	√	√	√	√
58[a]	7.693	$[M-H]^-$	499.30665	0.38	$C_{30}H_{44}O_6$	455.31589, 423.28726, 409.31454, 247.75471, 229.04976, 139.07742, 100.93333	11-Deoxocucurbitacin I	√	√	√	√

Table 2 (*Contd.*)

Peak no.	RT (min)	Reference ion	*m/z*	Diff. (ppm)	Formula	Fragment ions (*m/z*)	Identification	QJ	CJQJ	MHQJ	XQJ
59	7.731	$[M-H]^-$	403.1185	−0.53	$C_{24}H_{20}O_6$	388.09537, 357.11511, 319.18762, 298.02972, 229.04922, 217.0874, 201.05643, 151.96779	Tribenzoin		√	√	
60	7.777	$[M-H]^-$	343.22738	−1.42	$C_{22}H_{32}O_3$	315.25467, 297.24362, 287.22321, 269.21329, 229.02066, 201.11327, 187.0975, 139.11281	Medroxyprogesterone			√	√
61	7.81	$[M-H]^-$	265.12357	0.62	$C_{18}H_{18}O_2$	247.1111, 229.04784, 117.73475, 111.88721	Magnolol	√			
62	7.86	$[M-H]^-$	285.04067	0.72	$C_{15}H_{10}O_6$	267.19684, 257.0451, 241.21785, 229.04376, 223.20557, 174.75847, 112.1844	Kaempferol		√		√
63	7.89	$[M-H]^-$	235.09763	0.54	$C_{13}H_{16}O_4$	229.02434, 199.85121, 189.85136, 176.08412, 163.64058, 134.89507	4-(3-Hydroxy-1-buten-1-yl)-3-methoxy-5-methylbenzoic acid	√			√
64	7.956	$[M-H]^-$	299.20142	−0.77	$C_{20}H_{28}O_2$	281.21219, 255.23141, 237.22255, 229.03529, 197.14368, 173.10722, 157.88564	Tretinoin			√	
65[a]	7.991	$[M-H]^-$	471.34794	−0.05	$C_{30}H_{48}O_4$	318.6073, 229.02156, 195.29953, 152.99611, 144.25693	Colosolic acid	√	√	√	√
66	8.071	$[M-H]^-$	503.33821	0.78	$C_{30}H_{48}O_6$	473.32901, 459.35007, 441.33878, 425.30679, 341.26569, 279.23495, 237.15018, 152.18785	2,3,19,23-Tetrahydroxyolean-12-en-28-oic acid		√	√	√
67	8.241	$[M-H]^-$	315.19681	0.77	$C_{20}H_{28}O_3$	296.23151, 271.20706, 243.1758, 229.05154, 120.11904	15d-PGA2				√
68[a]	8.249	$[M-H]^-$	469.33264	0.66	$C_{30}H_{46}O_4$	451.32596, 425.34454, 383.35666, 280.06595, 229.03511	Enoxolone	√	√	√	√
69[a]	8.341	$[M-H]^-$	455.35293	0	$C_{30}H_{48}O_3$	393.27533, 375.2677, 327.8494, 279.23322, 257.23996, 229.04012, 175.06078, 114.02003	β-Boswellic acid	√	√	√	√
70	8.471	$[M-H]^-$	301.18104	0.4	$C_{19}H_{26}O_3$	272.23172, 254.2231, 229.03221, 218.09511, 204.11674, 189.09203	2-Methoxyestradiol				√
71[a]	8.702	$[M-H]^-$	487.3429	0.08	$C_{30}H_{48}O_5$	469.33502, 443.34897, 425.34457, 369.31638, 353.28592, 229.0455	Arjunic acid	√	√	√	√
72[a]	8.734	$[M-H]^-$	455.35301	−0.05	$C_{30}H_{48}O_3$	437.3428, 379.05026, 365.32156, 297.94916, 285.42944, 229.03523, 160.8452	Oleanolic acid	√	√	√	√
73[a]	8.762	$[M-H]^-$	323.25943	0.75	$C_{20}H_{36}O_3$	295.26431, 265.25485, 238.83621, 229.02774, 165.12883, 125.39846	Labdanolic acid	√	√	√	√
74[a]	8.857	$[M-H]^-$	455.35294	−0.24	$C_{30}H_{48}O_3$	437.34277, 411.33011, 229.03543, 214.91963, 144.2729, 128.21217	Ursolic acid	√	√	√	√
75	9.112	$[M-H]^-$	485.32726	0.04	$C_{30}H_{46}O_5$	441.33749, 423.3273, 407.29764, 381.31573, 365.28815, 229.02361, 177.2758	Melaleucic acid (6CI)				√
76[a]	9.457	$[M-H]^-$	391.28268	−6.88	$C_{24}H_{40}O_4$	363.28711, 355.32327, 343.26233, 229.02185, 191.03249, 172.81613, 142.60358	Deoxycholic acid	√	√	√	√

Table 2 (*Contd.*)

Peak no.	RT (min)	Reference ion	*m/z*	Diff. (ppm)	Formula	Fragment ions (*m/z*)	Identification	QJ	CJQJ	MHQJ	XQJ
77[a]	9.53	$[M-H]^-$	453.33751	0.38	$C_{30}H_{46}O_3$	435.32776, 391.28375, 355.77863, 298.2478, 229.0423, 171.10327	Pinicolic acid	√	√	√	√
78[a]	9.602	$[M-H]^-$	469.33236	0.17	$C_{30}H_{46}O_4$	425.3428, 411.29059, 397.31042, 367.30142, 339.26877, 229.04851	18-β-Glycyrrhetinic acid	√	√	√	√
79[a]	9.664	$[M-H]^-$	453.33746	0.11	$C_{30}H_{46}O_3$	435.32736, 393.31696, 336.5705, 247.89471, 229.03549, 165.65364, 157.5334	Glycyrrhetaldehyde	√	√	√	√
80[a]	10.104	$[M-H]^-$	471.34791	−0.03	$C_{30}H_{48}O_4$	441.33719, 427.35895, 413.30646, 397.35464, 341.28409, 251.1653, 229.02982, 191.1433, 152.99596	Bourjotinolone A (7CI)	√	√	√	√
81[a]	10.432	$[M-H]^-$	783.4906	0.74	$C_{42}H_{72}O_{13}$	737.48523, 600.46472, 575.43262, 484.41187, 323.10037, 221.06688, 179.05632, 161.04568	Ginsenoside F2	√	√	√	√
82[a]	12.115	$[M-H]^-$	455.353	0.02	$C_{30}H_{48}O_3$	437.34271, 408.33716, 383.33078, 312.17545, 229.02316, 175.14978	3-Hydroxyurs-12-en-23-oic acid	√	√	√	√
83[a]	13.162	$[M-H]^-$	457.36858	−0.21	$C_{30}H_{50}O_3$	439.36069, 399.32846, 333.66791, 293.06851, 229.04471, 153.84746, 120.77197	Soyasapogenol B	√	√	√	√
84[a]	13.44	$[M-H]^-$	439.35802	−0.25	$C_{30}H_{48}O_2$	313.57648, 263.74875, 229.02229, 194.77271, 163.58023, 137.89886, 120.79447	Roburic acid	√	√	√	√
85	13.618	$[M-H]^-$	437.34251	0.02	$C_{30}H_{46}O_2$	419.33331, 365.32208, 361.2926, 345.65881, 229.03558, 152.99625, 127.24102	2,2′-Ethylidene-bis(4,6-di-*tert*-butylphenol)				√
86[a]	13.873	$[M-H]^-$	415.32155	−0.51	$C_{27}H_{44}O_3$	380.88861, 326.64908, 229.02951, 216.85457, 163.04048, 145.02974, 118.04257	Calcitriol	√	√	√	√
87[a]	13.951	$[M-H]^-$	441.33739	−0.07	$C_{29}H_{46}O_3$	402.7934, 383.35178, 355.32266, 260.00064, 243.20294, 229.04424, 193.1041, 163.04025, 145.02969	4-α-Methylzymosterol-4-carboxylate	√	√	√	√
88[a]	14.016	$[M-H]^-$	425.34254	0.16	$C_{29}H_{46}O_2$	407.33124, 379.39474, 363.36276, 349.29794, 238.83777, 229.03508, 152.10498, 134.22285	4-β-Methylzymosterol-4-carbaldehyde	√	√	√	√
89[a]	14.923	$[M-H]^-$	427.35806	−0.2	$C_{29}H_{48}O_2$	367.33734, 288.5162, 229.05119, 174.3268, 116.92864	(3β,24*R*,24′*R*)-fucosterol epoxide	√	√	√	√
90[a]	16.017	$[M-H]^-$	443.35303	−0.06	$C_{29}H_{48}O_3$	305.3432, 300.46295, 252.77142, 229.05147, 201.34343, 163.04047, 145.03, 139.7027, 118.04246	3-β-Hydroxy-4*β*-methyl-5*α*-cholest-7-en-4*α*-oic acid	√	√	√	√
91[a]	17.338	$[M-H]^-$	433.36878	0.19	$C_{28}H_{50}O_3$	397.36902, 389.37885, 322.08398, 258.96381, 229.03616, 180.1319, 152.99579, 146.96379	6-Deoxoteasterone	√	√	√	√

Table 2 (*Contd.*)

Peak no.	RT (min)	Reference ion	*m/z*	Diff. (ppm)	Formula	Fragment ions (*m/z*)	Identification	QJ	CJQJ	MHQJ	XQJ
92[a]	18.95	$[M-H]^-$	471.38424	−0.25	$C_{31}H_{52}O_3$	417.68497, 300.66922, 229.03661, 163.04039, 145.02951, 118.04212	(22*S*,24*R*)-24-Methyllanosta-8-en-22,28-epoxy-3β,28*α*-diol	√	√	√	√
93	20.902	$[M-H]^-$	485.39998	−0.07	$C_{32}H_{54}O_3$	440.36252, 397.52771, 344.73618, 302.97443, 229.05005, 145.02914, 116.92834	6-Deoxy-16-β-*O*-acetyl-leucotylin	√	√		

[a] The common compounds identified from *Gentianae Macrophyllae* Radix.

quality control (QC) sample was prepared by mixing an equal volume of each sample solution.

2.3. LC-MS conditions

The LC analysis was run on a Hypersil GOLDTM Aq-C18 column (20 × 2.1 mm, 1.9 μm) (Thermo Sencitific, MA, USA) with a Vanquish™ Flex UPLC system at 30 °C, using 0.01% formic acid (A) and acetonitrile (B) as the mobile phase at a flow rate of 0.3 mL min^{-1}. The gradient elution conditions were as follows: 5–30% B (0–1 min), 30–40% B (1–5 min), 40–90% B (5–6 min), 90–95% B (6–13 min), 95% B (13–21 min), and 5% B (21–24 min). The injection volume was 4 μL. MS analysis (qualitative analysis) was performed on a Orbitrap Exploris 120 in negative ion mode with a full scan MS spectrum over the *m/z* range 150–1000, using ion spray voltage of 2.5 kV, sheath gas of 50 Arb, aux gas of 10 Arb, sweep gas of 1 Arb, ion transfer tube temp of 325 °C, and vaporizer temp 350 °C. The orbitrap resolution of full scan MS was 60 000 and MS^2 was 15 000, and HCD Collision Energies (%) was kept at 30%.

2.4. HPLC analysis

2.4.1. HPLC conditions. The HPLC analysis was conducted on an Agilent 1260 Infinity II HPLC system equipped with a binary pump, an autosampler, a thermostated column compartment, and a diode array detector (Agilent Technologies, Santa Clara, CA, USA). The compounds were separated on a Waters Atlantis® T3-C18 column (4.6 × 50 mm, 5 μm, Waters™, MA, USA) at 28 °C. The mobile phase consisted of 0.04% aqueous formic acid (A) and methanol (B) using a gradient program of 20–25% (B) in 0–13 min, 25–35% (B) in 13–20 min, and 35% (B) in 20–25 min. The flow rate was 1.0 mL min^{-1}. The detection wavelength was 240 nm.

2.4.2. HPLC method validation. The precision of the HPLC analysis method was obtained by injecting six replicates. Six collateral sample solutions were applied to evaluate the repeatability of the approach. The durability was evaluated by analyzing the same sample solution and mix standards using three different columns, including the Waters Atlantis® T3-C18 column (4.6 × 250 mm, 5 μm), Agilent 5 TC-C18 (Agilent Technologies, Santa Clara, CA, USA) and YMC-Pack ODS-A (5 μm, 4.6 × 150 mm, YMC CO., Ltd., Kyoto 600-8106, Japan) in two HPLC systems (Agilent HPLC 1260 II and Shimadzu LC-40 D), respectively. The precision, repeatability, stability, and durability were measured by Relative Standard Deviation (RSD) values of Relative Peak Area (RPA) and Relative Retention Time (RRT). Signal-to-noise ratios (S/N) of 3 and 10 as the standard for Limits of Detection (LOD) and Limits of Quantification (LOQ), respectively. In order to evaluate the recovery, the standard with known concentration was added to the accurately weighed sample in terms of the three concentrations of the high, medium, and low, and prepared in parallel with triplicates according to the sample preparation method. Recovery was then calculated as follows:

$$\text{Recovery } (\%) = \frac{m_1 - m_2}{m_3} \times 100\%$$

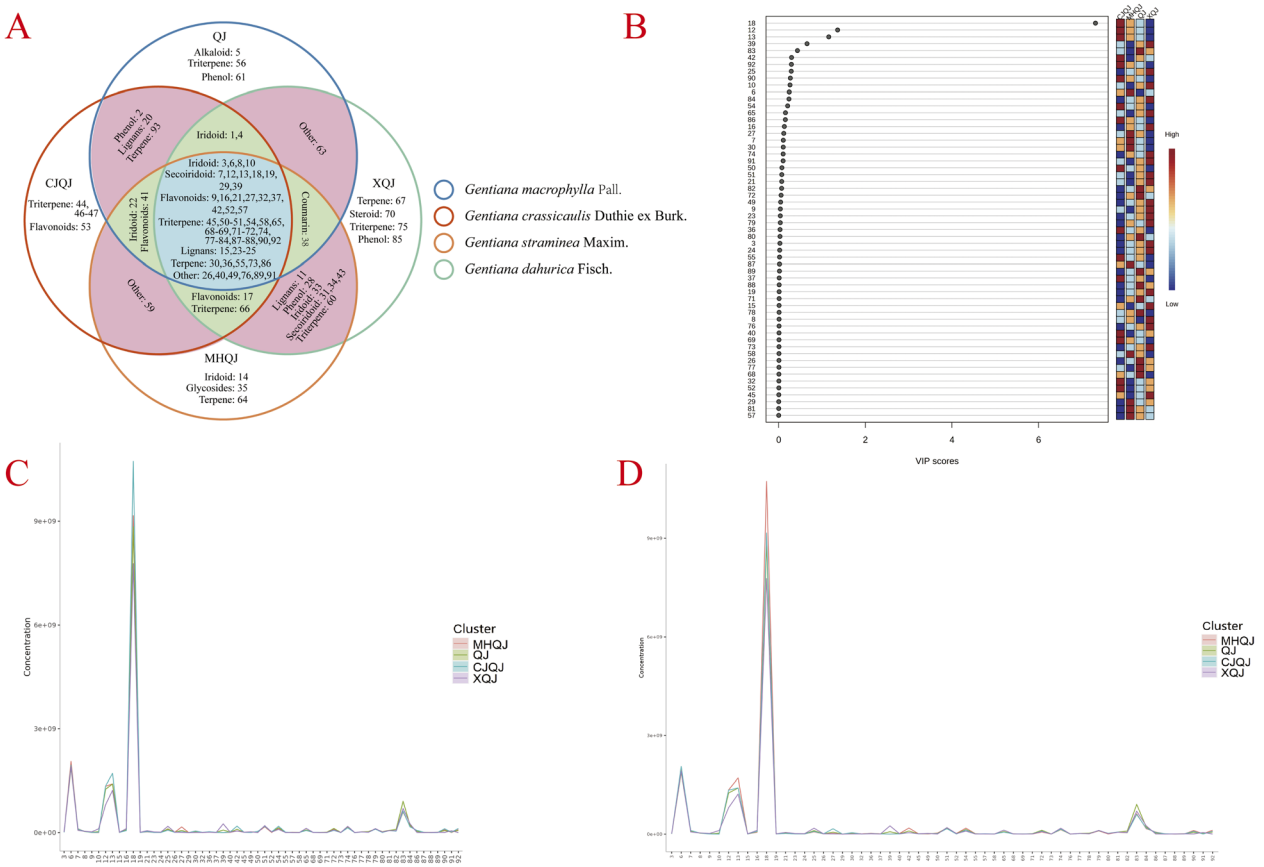

Fig. 3 The analysis of LC-MS data (A: the common peaks in Gentianae Macrophyllae Radix; B: the VIP score of 58 common peaks; C: K-means clustering; D: SOM).

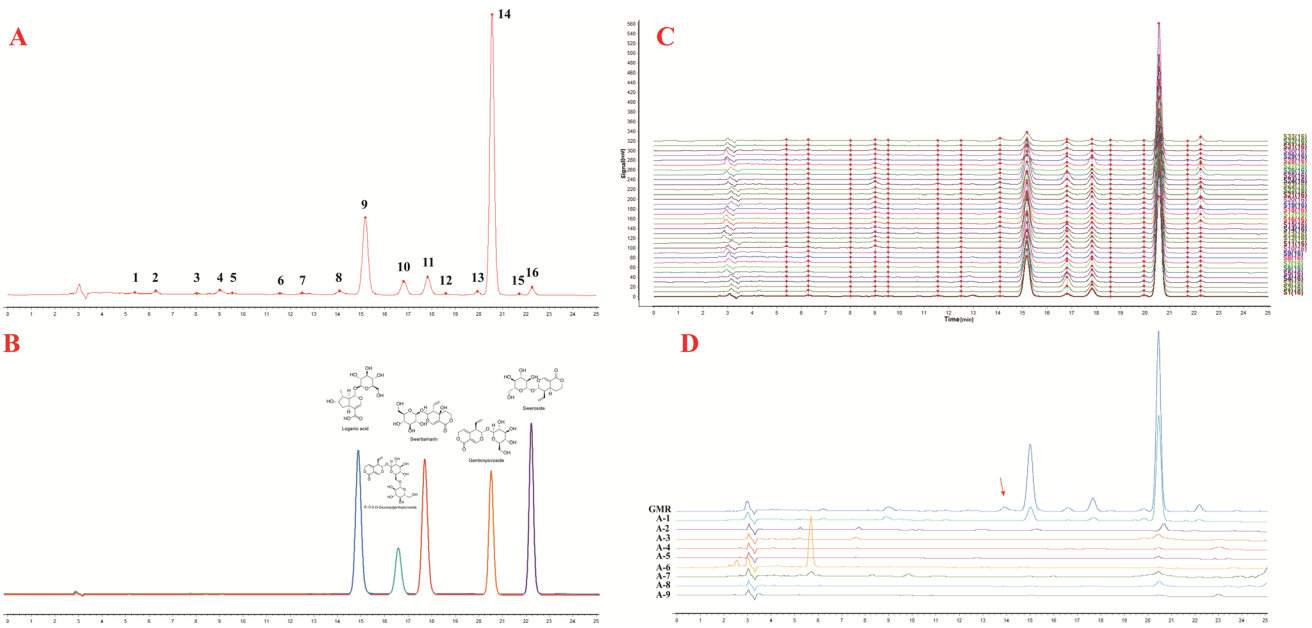

Fig. 4 The HPLC chromatograms of Gentianae Macrophyllae Radix (A) and mixed standards (B), the fingerprint of Gentianae Macrophyllae Radix (C), and comparison of Gentianae Macrophyllae Radix and its adulterants (D).

In which, m_1, m_2, and m_3 were the amount obtained, the half original amount in the sample, and the amount spiked into the sample, respectively.

2.4.3. SSDMC method development. Five reference standards at known concentrations were prepared as a mixed standard stock solution, which was then diluted to six different concentrations to obtain calibration curves for the quantitative analysis of the sample. SSDMC method was conducted by injecting one reference standard (gentiopicroside), and calculating the content of the other four components in sample solutions according to the response factor (F).

$$F = \frac{A_S/C_S}{A_i/C_i}$$

where A_i and A_S are the peak area of the corresponding compounds obtained from the sample solution and the peak area of gentiopicroside obtained from the standard solution. C_i and C_s are the concentration of components (mg mL^{-1}) to be measured in the test sample and the concentration of gentiopicroside in the reference solution.

2.5. Data analysis

The MS/MS data was analyzed by Freestyle 1.8 and Compounds discover 3.3 software. The compounds were identified by mzVault, mzCloud, ChemSpider, and Mass List Search. The VIP scores, K-means Clustering, SOM, and Pearson correlations were analyzed by Metaboanalyst 5.0. The fingerprint was generated by the Similarity Evaluation System for

Table 3 The precision, repeatability, and stability of the analysts

	Precision ($n = 6$)		Repeatability ($n = 6$)		Stability ($n = 8$)	
	RRT	RRA	RRT	RRA	RRT	RRA
Analyst	RSD (%)	RSD (%)	RSD (%)	RSD (%)	RSD (%)	RSD (%)
Loganic acid	0.10	0.09	0.28	1.82	0.75	1.63
6′-*O*-β-D-Glucosylgentiopicroside	0.10	0.31	0.30	2.23	0.66	1.68
Swertiamarine	0.07	0.30	0.26	2.12	0.54	1.59
Gentiopicroside	0.04	0.11	0.18	2.12	0.31	1.73
Sweroside	0.03	0.30	0.14	2.19	0.23	1.71

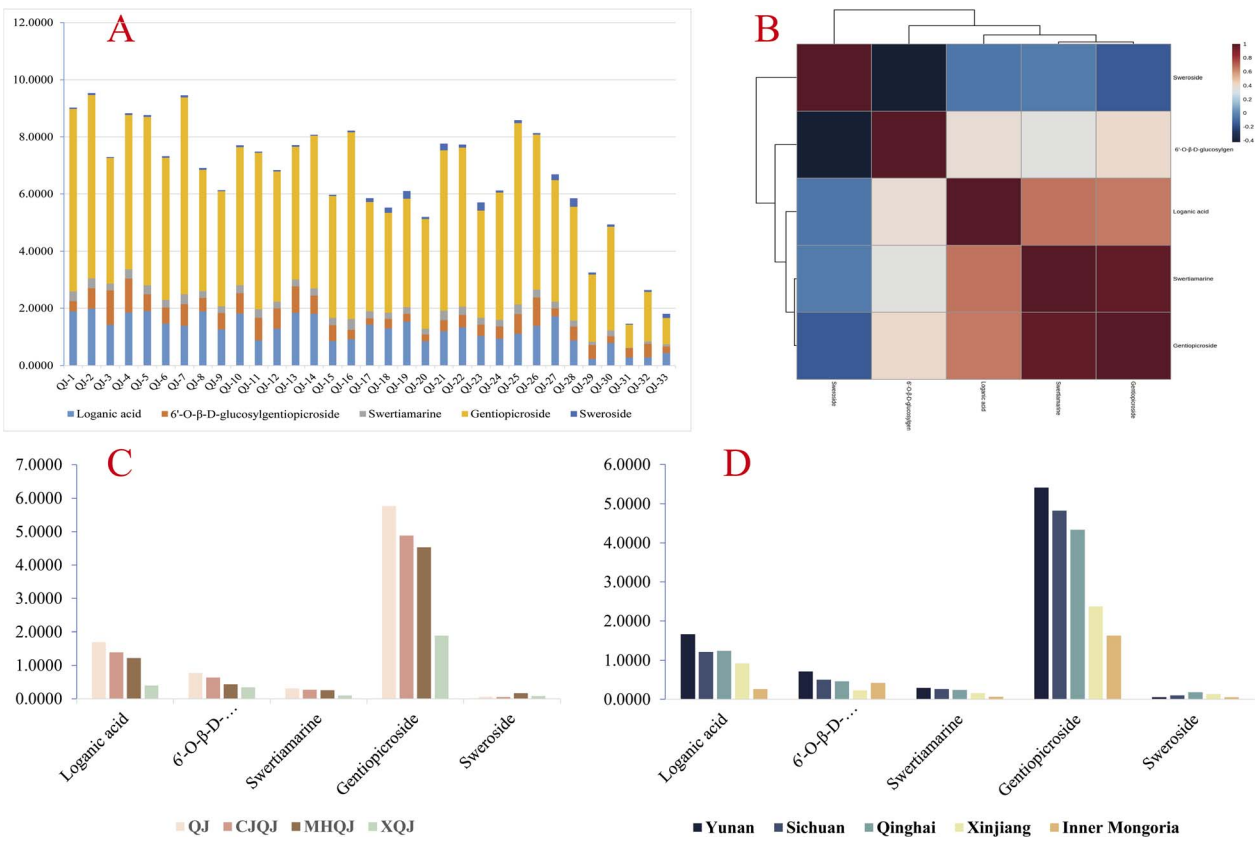

Fig. 5 The contents of five iridoids in Gentianae Macrophyllae Radix (A), the co-relationships of five active compounds (B), comparison of the contents of five active components among the Gentianae Macrophyllae Radix of the four species (C) and different regions (D).

Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2012A) software. PCA, PLS-DA, and HCA were obtained by SIMCA 14.1 software.

3. Results and discussion

3.1. LC-MS analysis

The response of Gentianae Macrophyllae Radix under the negative ion mode was better than the positive model in LC-MS analysis (Fig. S1†). The total ion chromatograms of QJ, CJQJ, MHQJ, XQJ, QC sample, and the adulterants are shown in Fig. 2 and S2.† The total ion chromatograms between Gentianae Macrophyllae Radix and its adulterants exhibit great difference in triterpenoid with retention time from 10 to 20 min (Fig. 2E). On the basis of reference standards, literature data,[8,22,23] and the inhouse and online database (inluding mzVault, mzCloud, ChemSpider, and Mass List Search with scores of more than 90), a total of 93 compounds were identified from Gentianae Macrophyllae Radix, including 9 iridoids, 10 secoiridoids, 12 flavonoids, 6 lignans, 38 terpenes, and 18 other types of compounds (Table 2). There were 58 common compounds in QJ, CJQJ, MHQJ, and XQJ (Fig. 3A). Their peak areas data was uploaded to Metaboanalyst 5.0 for statistical analysis (one factor) to screen out the differential components through VIP scores (Fig. 3B). Compounds **18** (gentiopicroside), 12 (6′-*O*-β-D-glucosylgentiopicroside), and **13** (swertiamarine) were the critical markers due to their high VIP scores. In addition, K-means calculation and SOM specified that compounds **6** (loganic acid), **18** (gentiopicroside), 12 (6′-*O*-β-D-glucosylgentiopicroside), **13** (swertiamarine), and **83** (soyasapogenol B) were the most critical components (Fig. 3C and D). Therefore, the HPLC analysis focused on these compounds.

3.2. HPLC analysis

After optimization, methanol-0.04% formic acid water was finally selected as the elution system (Fig. S3†) for HPLC analysis. Five of the peaks were identified to belong to loganic acid, 6′-*O*-β-D-glucosylgentiopicroside, swertiamarine, gentiopicroside, and sweroside by comparison with the reference standards (Fig. 4A and B). The precision, stability, and repeatability results are shown in Tables 3 and S1.† The durability result is shown in Table S2.† All the RSD values of the five compounds were less than 3.0%, which indicated this developed method was sensitive, precise, and robust.

All the collected samples were analyzed according to the HPLC method. Thereafter, the data was used to establish the fingerprints. As a result, 16 common peaks were observed in QJ, CJQJ, MHQJ, and XQJ (Fig. 4C). Loganic acid, 6′-*O*-β-D-

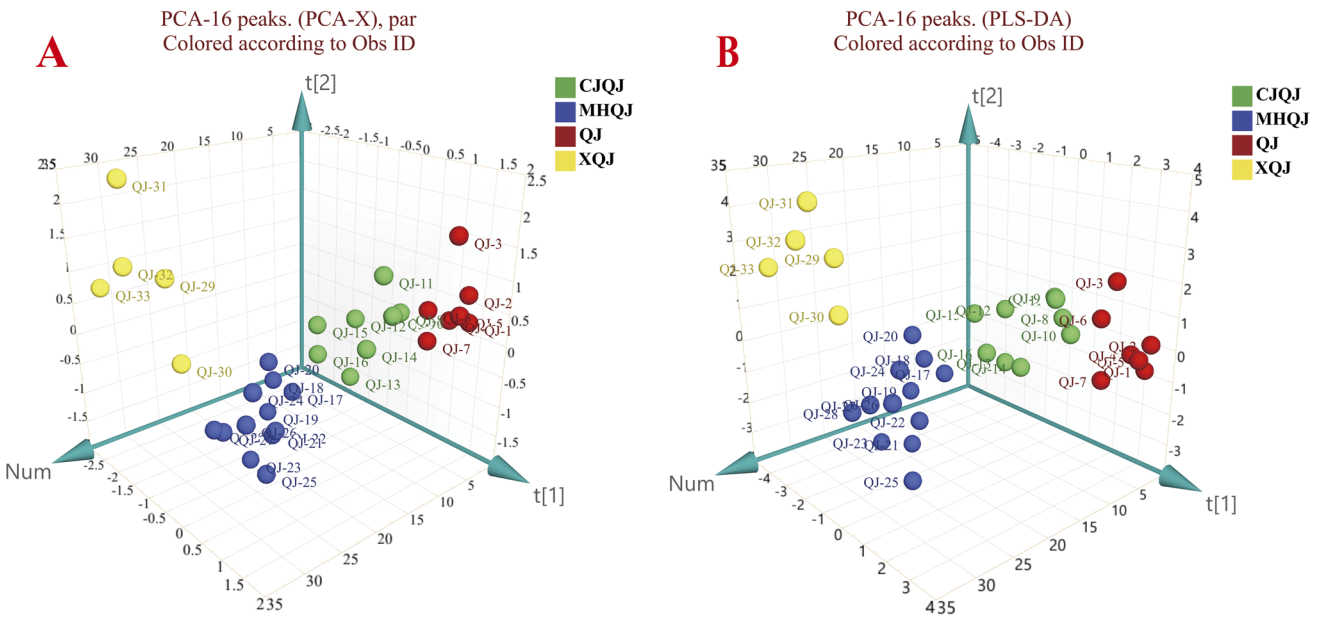

Fig. 6 PCA (A) and PLS-DA (B) analyses.

Table 4 The calibration curves of the analysts

Analyst	RT (min)	Calibration curve	R^2	Linear range (mg mL^{-1})	LOD (mg mL^{-1})	LOQ (mg mL^{-1})
Loganic acid	14.91	$y = 7472.7x + 1.9765$	0.9996	0.0065625–0.21	1.09×10^{-5}	3.62×10^{-5}
6′-*O*-β-D-Glucosylgentiopicroside	16.51	$y = 3007.1x - 0.5279$	0.9995	0.004688–0.15	2.81×10^{-5}	9.36×10^{-5}
Swertiamarine	17.56	$y = 8048.6x + 5.1576$	0.9994	0.001894–0.0606	1.00×10^{-5}	3.34×10^{-5}
Gentiopicroside	20.34	$y = 5858.9x + 3.1785$	0.9996	0.025313–0.81	1.14×10^{-5}	3.79×10^{-5}
Sweroside	22.06	$y = 7385.1x - 1.0209$	0.9994	0.001656–0.053	8.35×10^{-6}	2.78×10^{-5}

Table 5 The recovery of the analysts

Analytes	Level	Original (mg)	Spiked (mg)	Found (mg)	Average (%)	RSD (%)
Loganic acid	High	2.8498	2.1	3.684	104.52	1.157
	Medium		1.575	3.104	103.47	1.846
	Low		1.05	2.548	102.96	1.211
6′-*O*-β-D-Glucosylgentiopicroside	High	0.4512	0.46	0.695	101.44	1.368
	Medium		0.23	0.471	103.44	1.926
	Low		0.15	0.378	100.74	1.395
Swertiamarine	High	0.4905	0.505	0.752	100.26	1.199
	Medium		0.2525	0.508	102.09	1.464
	Low		0.101	0.367	105.93	1.564
Gentiopicroside	High	7.6598	4.536	8.487	101.45	1.169
	Medium		3.8475	7.754	101.00	1.822
	Low		1.9278	5.647	98.07	1.280
Sweroside	High	0.2691	0.212	0.356	102.83	1.191
	Medium		0.1378	0.271	99.62	1.853
	Low		0.0636	0.203	102.63	1.616

Table 6 Comparsion of the contents determined by calibration curve and SSDMC methods

	Loganic acid (mg mL^{-1})		6′-*O*-β-D-Glucosylgentiopicroside (mg mL^{-1})		Swertiamarine (mg mL^{-1})		Gentiopicroside (mg mL^{-1})	Sweroside (mg mL^{-1})	
No.	Calibration curve	SSDMC	Calibration curve	SSDMC	Calibration curve	SSDMC	Calibration curve	Calibration curve	SSDMC
QJ-1	0.1893	0.1872	0.0355	0.0354	0.035	0.032	0.6393	0.0045	0.0044
QJ-2	0.1985	0.1963	0.0721	0.0722	0.034	0.032	0.6421	0.0068	0.0067
QJ-3	0.1421	0.1406	0.1208	0.1210	0.023	0.022	0.4400	0.0030	0.0029
QJ-4	0.1846	0.1826	0.1196	0.1198	0.032	0.030	0.5406	0.0061	0.0060
QJ-5	0.1901	0.1880	0.0589	0.0590	0.031	0.029	0.5897	0.0064	0.0063
QJ-6	0.1459	0.1444	0.0565	0.0565	0.027	0.025	0.4982	0.0052	0.0051
QJ-7	0.1385	0.1370	0.0759	0.0760	0.035	0.032	0.6899	0.0073	0.0073
QJ-8	0.1891	0.1870	0.0476	0.0476	0.024	0.023	0.4250	0.0063	0.0062
QJ-9	0.1259	0.1246	0.0578	0.0579	0.023	0.022	0.4030	0.0040	0.0039
QJ-10	0.1814	0.1794	0.0718	0.0719	0.028	0.026	0.4835	0.0065	0.0065
QJ-11	0.0870	0.0861	0.0800	0.0801	0.030	0.028	0.5489	0.0038	0.0037
QJ-12	0.1284	0.1271	0.0711	0.0712	0.023	0.022	0.4566	0.0046	0.0045
QJ-13	0.1840	0.1819	0.0928	0.0930	0.024	0.023	0.4654	0.0056	0.0055
QJ-14	0.1810	0.1790	0.0638	0.0638	0.025	0.024	0.5344	0.0035	0.0034
QJ-15	0.0849	0.0841	0.0558	0.0559	0.025	0.024	0.4274	0.0042	0.0041
QJ-16	0.0911	0.0902	0.0330	0.0329	0.038	0.035	0.6545	0.0058	0.0057
QJ-17	0.1425	0.1410	0.0226	0.0225	0.025	0.023	0.3830	0.0135	0.0135
QJ-18	0.1297	0.1284	0.0332	0.0331	0.021	0.020	0.3502	0.0179	0.0180
QJ-19	0.1531	0.1514	0.0275	0.0275	0.024	0.022	0.3795	0.0267	0.0269
QJ-20	0.0835	0.0827	0.0242	0.0241	0.020	0.019	0.3851	0.0063	0.0063
QJ-21	0.1192	0.1180	0.0396	0.0396	0.033	0.031	0.5625	0.0231	0.0233
QJ-22	0.1325	0.1312	0.0441	0.0441	0.029	0.027	0.5574	0.0111	0.0111
QJ-23	0.1026	0.1016	0.0397	0.0397	0.024	0.022	0.3759	0.0287	0.0290
QJ-24	0.0933	0.0924	0.0430	0.0430	0.023	0.021	0.4458	0.0071	0.0071
QJ-25	0.1103	0.1091	0.0694	0.0695	0.034	0.032	0.6359	0.0104	0.0104
QJ-26	0.1383	0.1368	0.0999	0.1001	0.028	0.026	0.5438	0.0054	0.0053
QJ-27	0.1717	0.1698	0.0278	0.0277	0.024	0.022	0.4260	0.0200	0.0201
QJ-28	0.0875	0.0867	0.0484	0.0484	0.020	0.019	0.3991	0.0295	0.0298
QJ-29	0.0230	0.0230	0.0480	0.0480	0.011	0.011	0.2360	0.0073	0.0073
QJ-30	0.0783	0.0775	0.0240	0.0239	0.020	0.019	0.3645	0.0081	0.0080
QJ-31	0.0282	0.0281	0.0320	0.0320	0.001	0.002	0.0810	0.0035	0.0034
QJ-32	0.0288	0.0287	0.0463	0.0463	0.008	0.008	0.1744	0.0061	0.0060
QJ-33	0.0427	0.0424	0.0232	0.0232	0.007	0.007	0.0926	0.0142	0.0143

glucosylgentiopicroside, swertiamarine, gentiopicroside, and sweroside were relatively abundant in Gentianae Macrophyllae Radix compared with it adulterates, which are the key ingredients for the authentication (Fig. 4D). The content of active compounds is the linchpin for distinguishing Gentianae Macrophyllae Radix of the four species. Gentiopicroside had a significant relationship with swertiamarine ($p < 0.01$), and sweroside had a significant negative relationship with 6′-*O*-β-D-glucosylgentiopicroside ($p < 0.05$) (Table S3† and Fig. 5B). The average content of each component in XQJ is far lower than QJ, CJQJ, and MHQJ, and the content of sweroside in MHQJ is the highest (Fig. 5C). In addition, the five active components of Gentianae Macrophyllae Radix in Yunnan, Sichuan, and Qinghai all show high content, and the content of sweroside in Gentianae Macrophyllae Radix of Qinghai is the highest (Fig. 5D). In order to further confirm the findings, the peak area data of 16 common compounds was used for PCA and PLS-DA, which could also distinguish QJ, CJQJ, MHQJ, and XQJ (Fig. 6).

SSDMC method based on the optimized HPLC was developed for their simultaneous detection of the compounds. The calibration curves, linear ranges, LOD, and LOQ of the analytes are shown in Table 4. The average relative response factors (*F*) for loganic acid, 6′-*O*-β-D-glucosylgentiopicroside, swertiamarine, and sweroside were 0.78, 1.97, 0.67, and 0.81, with an RSD of 1.31%, 0.89%, 0.08%, and 1.76%, respectively (Table S4†). Additionally, the recovery of loganic acid, 6′-*O*-β-D-glucosylgentiopicroside, swertiamarine, gentiopicroside, and sweroside was 102.96–104.52%, 100.74–103.44%, 100.26–105.93%, 98.07–101.45%, and 99.62–102.83%, respectively (Table 5). Combined with the results from method validation in the HPLC fingerprint study, the described SSDMC approach proved to be robust, sensitive, precise, and accurate. As shown in Table 6, the results calculated by the SSDMC method showed no significant difference from the calibration curve method.

4. Conclusion

In this paper, a LC-Orbitrap-MS method was established to analyze the common or characteristic components of Gentianae Macrophyllae Radix originated from four species, which led to the identification of 93 components, including 58 common ones in the four species. It also proved that Gentianae Macrophyllae Radix mainly contains terpenes (iridoids and triterpenes), flavonoids, alkaloids, lignans, and sterols. The terpenes (with retention time between 10 to 20 min) were the characteristic compounds to identify Gentianae Macrophyllae Radix and its adulterants. The established HPLC fingerprint could also distinguish this medicine and its adulterants depended on the five critical compounds of loganic acid, 6′-*O*-β-D-glucosylgentiopicroside, swertiamarine, gentiopicroside, and sweroside. Another compound (peak 8) is also one of the specific components of Gentianae Macrophyllae Radix, but it has not been identified (Fig. 4D). In addition, HPLC combined with PCA and PLS-DA could identify QJ, CJQJ, MHQJ, and XQJ based on the content of 16 common peaks. The SSDMC method is also powerful for the determination of five main compounds. It is very important to select the authentic and high-quality medicinal materials because the level of the compounds is directly related to the clinical efficacy.[6,7,14,15,24,25]

In conclusion, the developed LC-Orbitrap-MS and HPLC strategy is of great importance for quality control and authentication of Gentianae Macrophyllae Radix. The further study is needed for the comparison of pharmacological effects of Gentianae Macrophyllae Radix of the four species and the impact of geographical and ecological environment on its chemicals.

Conflicts of interest

All the authors have declared no conflict of interest.

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